

CLAIMS

1. A method of producing a modified target enzyme having an altered performance profile, comprising the steps of:

- (a) providing a target enzyme;
- (b) analyzing said target enzyme to identify one or more regions or amino acid residues in the target enzyme for modification;
- (c) modifying said one or more regions or amino acid residues identified in the target enzyme so as to introduce a catalytic triad in the target enzyme, wherein said catalytic triad includes a first member comprising an amino acid residue or chemical group which acts as a proton donor, a second member which is equivalent to histidine 200 in the sequence of Bacillus cellulase 103 (SEQ ID NO:2), and a third member which is an equivalent to serine 227 in the sequence of Bacillus cellulase 103 (SEQ ID NO:2); and
- (d) selecting a modified target enzyme having an altered performance profile as compared to the target enzyme of (a).

2. The method of claim 1 wherein said first, second and third members of the catalytic triad of step (c) include amino acid residues equivalent to glutamine 139, histidine 200 and serine 227, respectively, in the sequence of Bacillus cellulase 103 (SEQ ID NO:2).

3. The method of claim 1 wherein said target enzyme is a cellulase.

4. The method of claim 1 wherein said target enzyme is a hydrolase.

5. The method of claim 1 wherein said altered performance profile is an altered pH profile.

6. The method of claim 5 wherein said target enzyme has an acidic pH profile and said modified target enzyme has an alkaline pH profile.

7. The method of claim 1 wherein said first member of the catalytic triad is a glutamate or aspartate residue.

8. The method of claim 1 wherein said third member of the catalytic triad is a serine, threonine or aspartate residue.

9. A method of producing a modified target enzyme having an altered pH profile, comprising the steps of:

- (a) providing a target enzyme having an acidic pH profile;
- (b) analyzing said target enzyme to identify one or more regions or amino acid residues in the target enzyme for modification;
- (c) modifying said one or more regions or amino acid residues identified in the target enzyme so as to introduce a catalytic triad in the target enzyme, wherein said catalytic triad includes a first member comprising an amino acid residue or chemical group which acts as a proton donor, a second member which is equivalent to histidine 200 in the sequence of Bacillus cellulase 103 (SEQ ID NO:2), and a third member which is an equivalent to serine 227 in the sequence of Bacillus cellulase 103 (SEQ ID NO:2); and
- (d) selecting a modified target enzyme having an alkaline pH profile as compared to the acidic pH profile of the target enzyme of (a).

10. The method of claim 9 wherein said target enzyme is a hydrolase.

11. The method of claim 9 wherein said first, second and third members of the catalytic triad of step (c) include amino acid residues equivalent to glutamine 139, histidine 200 and serine 227, respectively, in the sequence of Bacillus cellulase 103 (SEQ ID NO:2).

12. The method of claim 9 wherein said first member of the catalytic triad is a glutamate or aspartate residue.

13. The method of claim 9 wherein said third member of the catalytic triad is a serine, threonine or aspartate residue.

14. A method of producing a modified target enzyme having an altered pH profile, comprising the steps of:

- (a) providing a target enzyme having an acidic pH profile;
- (b) analyzing said target enzyme to identify one or more regions or amino acid residues in the target enzyme for modification;
- (c) modifying said one or more regions or amino acid residues identified in the target enzyme so as to introduce a catalytic triad in the target enzyme, wherein said catalytic triad includes a first member comprising a proton donor equivalent to Glutamate 139 in the Bacillus cellulase 103 sequence (Figure 3A-3E, SEQ ID NO:2), a second member comprising a residue equivalent to Histidine 200 in the Bacillus cellulase 103 sequence (Figure 3A-3E, SEQ ID NO:2), and a third member comprising a water molecule which functions in acid/base catalysis; and
- (d) selecting a modified target enzyme having an alkaline pH profile as compared to the acidic pH profile of the target enzyme of (a).

15. A method of producing a modified target enzyme having an altered performance profile, comprising the steps of:

- (a) providing a target enzyme;
- (b) analyzing said target enzyme to identify one or more regions or amino acid residues in the target enzyme for modification so as to introduce a catalytic triad, wherein said catalytic triad includes a first member comprising an amino acid residue or chemical group which acts as a proton donor, a second member which is equivalent to histidine 200 in the sequence of Bacillus cellulase 103 (SEQ ID NO:2), and a third member which is an equivalent to serine 227 in the sequence of Bacillus cellulase 103 (SEQ ID NO:2);
- (c) genetically modifying DNA encoding said one or more regions or amino acid residues identified in the target enzyme so as to create a library of modified target enzymes having mutations; and
- (d) selecting a modified target enzyme from said library having an altered performance profile as compared to the target enzyme of (a).

16. The method of claim 15 wherein said target enzyme is a cellulase.

17. The method of claim 15 wherein said target enzyme is a hydrolase.

18. The method of claim 15 wherein said altered performance profile is an altered pH profile.

19. The method of claim 18 wherein said altered pH profile is an alkaline pH profile.

20. A library of modified target enzymes produced in accordance with the method of claim 1 or claim 9.

21. A library of modified target enzymes produced in accordance with the method of claim 14 or claim 15.

22. A method of making a modified target enzyme having an altered performance profile, comprising the steps of:

- (a) providing a target enzyme;
- (b) analyzing said target enzyme to identify one or more regions or amino acid residues in the target enzyme for modification;
- (c) modifying said one or more regions or amino acid residues identified in the target enzyme so as to introduce a catalytic triad in the target enzyme, wherein said catalytic triad includes a first member comprising an amino acid residue or chemical group which acts as a proton donor, a second member which is equivalent to histidine 200 in the sequence of Bacillus cellulase 103 (SEQ ID NO:2), and a third member which is an equivalent to serine 227 in the sequence of Bacillus cellulase 103 (SEQ ID NO:2);
- (d) selecting a modified target enzyme having an altered performance profile as compared to the target enzyme of (a);
- (e) providing DNA encoding the selected modified target enzyme of (d) in a vector;
- (f) expressing the DNA of (e) so as to produce the selected modified target enzyme.

23. An isolated, modified target enzyme comprising a polypeptide genetically modified to comprise a catalytic triad that

alters the pH profile of the polypeptide, wherein said catalytic triad comprises a first member, a second member and a third member and said first member is a proton donor, said second member is equivalent to the Histidine 200 residue in the Bacillus cellulase 103 sequence (SEQ ID NO:2), and said third member is equivalent to the Serine 227 residue in the Bacillus cellulase 103 sequence (SEQ ID NO:2).

24. The modified target enzyme of claim 23 wherein said pH profile is an alkaline pH profile.

25. The modified target enzyme of claim 23 wherein the genetic modification in the polypeptide comprises a substitution, deletion or addition of amino acid residue(s) equivalent to one or more of amino acid residues Serine 227, Histidine 200, or Glutamate 139 in the Bacillus cellulase 103 sequence (SEQ ID NO:2).

26. The modified target enzyme of claim 23 wherein said enzyme is a cellulase.

27. The modified target enzyme of claim 23 wherein said enzyme is a hydrolase.

28. A nucleic acid molecule comprising DNA encoding the modified target enzyme of claim 23.

29. A vector comprising the nucleic acid of claim 28.

30. A host cell comprising the vector of claim 29.

31. The host cell of claim 30 which is a prokaryotic cell.

32. A method for making a modified target enzyme, comprising the steps of:

culturing the host cell of claim 30 transformed with a replicable expression vector comprising the DNA encoding a modified target enzyme and operably linked to a control sequence capable of effecting expression of the modified target enzyme in the host cell

and recovering and/or purifying the expressed modified target enzyme.

33. A modified target enzyme produced in accordance with the method of claim 1.

34. A modified target enzyme produced in accordance with the method of claim 9.

35. A modified target enzyme produced in accordance with the method of claim 14.

36. A modified target enzyme produced in accordance with the method of claim 15.

37. A detergent comprising the modified target enzyme of claim 23.

38. A composition comprising the modified target enzyme of claim 23 and a carrier.

39. The composition of claim 38 comprising a therapeutically acceptable carrier.